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(21) International Application Number: PCT/EP93/01575 (22) International Filing Date: 21 June 1993 (21.06.93) (30) Priority data: PD92A000110 19 June 1992 (19.06.92) IT (71) Applicant (for all designated States except US): M.U.R.S.T. ITALIAN MINISTRY FOR UNIVERSITIES AND SCIENTIFIC AND TECHNOLOGICAL RESEARCH [IT/IT]; Lungotevere Thaon de Revel, 39, I-Rome (IT). (72) Inventors; and (75) Inventors/Applicants (for US only) : PREHM, Peter [DE/DE]; In der Weede 75, D-48163 Münster (DE). O'REGAN, Michael [IT/IT]; Via Nazareth, 15, I-35100 Padova (IT). CALLEGARO, Lanfranco [IT/IT]; Via Bravi, 35, I-35020 Ponte di Brenta (IT). LELLIG, Sabine [DE/DE]; In der Weede 75, D-48163 Münster (DE).	(74) Agent: VOSSIUS & PARTNER (NO.31); Siebertstrasse 4, P.O. Box 86 07 67, D-8000 Munich 86 (DE). (81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 29 September 1994 (29.09.94)	
(54) Title: PRODUCTION OF HYALURONIC ACID BY TRANSFORMED MICROORGANISMS		
(57) Abstract <p>An isolated DNA fragment comprising a nucleotide sequence encoding hyaluronic acid synthase is provided. Also provided are recombinant expression vectors comprising this sequence, capable of expressing hyaluronic acid synthase in prokaryotic and eukaryotic cells, as well as host cells harboring such expression vectors. Such cells are capable of producing hyaluronic acid synthase, hyaluronic acid, or both, which can be recovered from these cells, the culture medium, or both. Also provided are a method of producing hyaluronic acid synthase, methods of producing hyaluronic acid both <i>in vivo</i> and <i>in vitro</i>, and hyaluronic acid produced by these methods. In the case of <i>in vitro</i> production, the molecular weight of hyaluronic acid can be varied by varying the time of incubation of hyaluronic acid precursors in the presence of hyaluronic acid synthase and/or the reaction conditions. Lastly, a method of purifying hyaluronic acid synthase from cultured cells is also provided.</p>		

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INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/EP 93/01575

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/52 C12N9/00 C12P19/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 03559 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 21 March 1991 cited in the application see the whole document ---	1-23
A	THE BIOCHEMICAL JOURNAL, vol.267, no.1, 1 April 1990, LONDON, UK pages 191 - 196 A. MAUSOLF ET AL. 'Shedding of hyaluronate synthase from Streptococci' cited in the application ---	
A	THE BIOCHEMICAL JOURNAL, vol.235, no.3, 1 May 1986, LONDON, UK pages 887 - 889 P. PREHM AND A. MAUSOLF 'Isolation of streptococcal hyaluronate synthase' cited in the application --- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

26 April 1994

Date of mailing of the international search report

24-08-1994

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 93/01575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE BIOCHEMICAL JOURNAL, vol.289, no.1, 1 January 1993, LONDON, UK pages 179 - 184 M. LANSING ET AL. 'Hyaluronate synthase: cloning and sequencing of the gene from Streptococcus sp.' -----	1-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 93/01575

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1- Claims 1-23 : Method for the production of hyaluronic acid synthase.
- 2- Claims 24-25 : Method for producing hyaluronic acid of varying molecular weight.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 - 23.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

information on patent family members

PCT/EP 93/01575

**Patent document
cited in search report**

Publication date

Patent family member(s)

Publication date

WO-A-9103559

21-03-91

US-A-

5015577

14-05-91

AU-A-

6342990

08-04-91